REFERENCE LABORATORY GENETICS

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GENETIC STUDY OF ONCOLOGICAL DISEASES (OncoRef Global®) BY MASSIVE SEQUENCING (NGS)

Request No.:	**000**		
Client:	-		
Analysis code:	57650		
Patient Name:	**xxx**		
Date of Birth:	21/02/1960	**Patient Ref.:**	xxx
Gender:	Female	**Sample Type:**	Whole blood

Clinical information: Family history of cancer, brother who died of malignant melanoma at age 25, young sister who died from a tumor of the ceruminous glands.

RESULT AND INTERPRETATION

No pathogenic or likely pathogenic variants have been detected in the sequence of the genes analysed.

The presence of a heterozygous variant of uncertain clinical significance (VUS) has been identified. (See Recommendations)

The complete list of studied genes is available in Annex 1. (Methodology)

The list of reported genes and coverage details is available in Table 1. (Methodology)

Gene	Variant*	Zygosity	Inheritance pattern	Classification^
CHEK2	NM_007194.3: **c.1510G>C** **p.Glu504Gln**	Heterozygosis	Autosomal Dominant	**VUS**

* Nomenclature according to HGVS v15.11
^ Based on the recommendations of the *American College of Medical Genetics and Genomics (ACMG)*

The ***CHEK2*** variant **c.1510G>C (p.Glu504Gln)** is a *missense* that predicts an conserved amino acid change from Glutamic acid to Glutamine at position 504 of the protein. It is described in the ClinVar database (142479) or variant of uncertain clinical significance based on the scientific literature, where this variant has been reported as a variant of uncertain clinical significance. This variant was found in a patient with cancer (unspecified type) and in a patient with Lynch syndrome. (PMID: 25318351, 28577310). It has not been reported in the HGMD database. However, it appears in the gnomAD population frequency database

(0.0038%). All bioinformatic predictors (SIFT, Mutation Taster and Polyphen-2) estimate that the change would have a benign effect.
Based on these data, the variant is classified as a **Variant of Uncertain Clinical Significance.**

Variants in the *CHEK2* gene have been associated with Li-Fraumeni Syndrome(OMIM: 609265) with an autosomal dominant inheritance pattern.

RECOMMENDATIONS

It is recommended that the patient and her relatives make an appointment at a hereditary cancer / genetic oncology unit if it has not yet been performed.

In order to establish co-segregation with the disease and thus the possible pathogenicity of a variant, it must be studied in parents and/or affected and unaffected relatives. In oncology, variants represent a risk factor. So the presence of the variant, by itself, in a healthy person does not mean that the person will suffer from cancer. Therefore, the possible informativity of co-segregation studies should be assessed on a case-by-case basis according to the family tree.

Genetic counselling should be offered to the patient by the prescriber physician. If additional information regarding the results or genetic counselling is required, the physician can contact our team at genetics@referencelaboratory.es.

METHODOLOGY

DNA extraction and quantitative and qualitative evaluation of the DNA obtained.

Capture and enrichment of exonic regions and flanking intronic areas of genes contained in the REFLAB MedExome (Roche) sequencing panel with the Roche NimbleGen SeqCap EZ HyperCap Library™ technology.

Massive sequencing with the NextSeq™(Illumina) sequencer.

Identification of the variants of interest in regards to the reference genome (hg19) after filtering, according to specific quality criteria. Annotation of the obtained variants with a specific bioinformatic software: Alamut Visual™ (Interactive Biosoftware), Ingenuity Variant Analysis™ (QIAGEN), Variant interpreter™ (Illumina) and VarAFT™. The used reference databases have been the population databases dbSNP, 1000genomes, EXAC and gnomAD; the clinical databases Human Gene Mutation Database (HGMD version 2019.3), ClinVar and LOVD; the disease specific databases, if applicable, and Reference Laboratory Genetics´own databases. The bioinformatic analysis to evaluate the possible impact of the variants of interest on the structure and functionality of the protein has been carried out with the bioinformatic programs Mutation Taster, SIFT and PolyPhen-2. These analyses are only a predictive tool; they were not experimentally proven.

The nomenclature used to define the variants follows the criteria of the *Human Genome Variation Society (HGVS)* (http://www.HGVS.org/varnomen).

Classification of variants based on the recommendations of the *American College of Medical Genetics and Genomics (ACMG)* (Richards S. *et al*., 2015). Only those variants that, based on current information, are considered pathogenic, likely pathogenic or of uncertain clinical significance, are reported. (The complete list of identified variants is available upon request).

The obtained average reading depth was 117,3x being > 20x in 98,4% of the regions analysed.

The reported INDEL variants are confirmed by Sanger sequencing.

LIMITATIONS: The results obtained do not exclude variants outside the analysed regions of the genome or genetic anomalies not detectable by massive sequencing such as large rearrangements, large deletions/duplications (Copy Number Variant; CNV), insertions / deletions of> = 10 nucleotides, variants in repetitive regions or with a high percentage of GC, and variants in genes with pseudogenes with highly homologous sequences.
It is not possible to rule out the presence of variants in other unanalysed genes.

Annex 1. List of studied genes

AIP, AKT1, ALK, APC, ATM, ATR, AXIN2, BAP1, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, BUB1B, CASR, CCND1, CDC73, CDH1, CDK4, CDKN1B, CDKN1C, CDKN2A, CEBPA, CEP57, CHEK2, CTC1, CTNNA1, CYLD, DDB2, DICER1, DIS3L2, DKC1, EGFR, EGLN1, EPCAM, ERCC1, ERCC2, ERCC3, ERCC4, ERCC5, EXT1, EXT2, EZH2, FAM175A, FAN1, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, FH, FLCN, GALNT12, GATA2, GPC3, GREM1, HNF1A, HNF1B, HOXB13, HRAS, IL1B, IL1RN, KIF1B, KIT, LIG4, LZTR1, MAD2L2, MAX, MC1R, MEN1, MET, MITF, MLH1, MLH3, MRE11, MRE11A, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NF2, NHP2, NOP10, NSD1, NTHL1, PALB2, PALLD, PDGFRA, PHOX2B, PIK3CA, PMS1, PMS2, POLD1, POLE, POLH, POT1, PRF1, PRKAR1A, PRSS1, PTCH1, PTCH2, PTEN, RAD50, RAD51, RAD51C, RAD51D, RB1, RECQL, RECQL4, RET, RHBDF2, RINT1, RNF43, RPS20, RUNX1, SBDS, SCG5, SDHA, SDHAF2, SDHB, SDHC, SDHD, SEC23B, SLC45A2, SLX4, SMAD4, SMARCA4, SMARCB1, SMARCE1, SPINK1, SPRED1, SRGAP1, STK11, SUFU, TERT, TINF2, TMEM127, TP53, TSC1, TSC2, TYR, UBE2T, VHL, WRAP53, WRN, WT1, XPA, XPC, XRCC2, XRCC3, YAP1,

Table 1. List of reported genes and coverage details

Gene	NM	10x %	Exons with coverage < 100%*
CHEK2	NM_007194	98,9	5 13

*Due to the current intrinsic limitations associated with massive sequencing technology, some gene exons analysed may be insufficiently covered. If it is considered appropriated by a medical specialist, it would be possible to sequence exons with coverage below 100% using the Sanger method or other alternative molecular technique.

IMPORTANT NOTE

The information contained in this report is based on current scientific knowledge and the results obtained from the application of the technology in this report, are detailed. Due to continuous advances, the documented information may be modified in the future as a result of the emergence of new scientific evidence.
The genetic/genomic studies carried out by Reference Laboratory S.A. are exclusively intended for qualified health professionals for their interpretation. The results obtained are not, per se, a medical consultation, diagnosis or treatment, nor should they be interpreted as such. Only a specialized professional can correctly interpret the results and offer a diagnosis or prescribe a treatment to a patient based on these. Consequently, no information obtained from our studies can be used to replace the advice and diagnosis of a specialized professional.

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